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FINAL REPORT

TOXICOLOGIC EVALUATION OF THE MIGRATION OF A PLASTICIZER,
DI(2-ETHYLHEXYL) PHTHALATE (DEHP) FROM VINYL PLASTICS.

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SUMMARY

The intravenous administration of DEHP solubilized by means of a number of different detergents leads to respiratory distress and death in rats. At autopsy the lungs are grossly enlarged, edematous, and hemorrhagic. Light and electron microscopic evaluation of the lungs indicate engorgement of the interalveolar septa with edema fluid and polymorphonuclear leucocytes, degranulation of the leukocytes, and progressive destruction of the endothelial and epithelial cells. Red blood cell structure was also seen to be altered. With the exception of this latter observation, all of these pathologic effects are identical to those seen in "shock lung", a disease state whose pathogenesis is not clearly understood at this time. Consistent with the conclusion that solubilized DEHP results in a syndrome of "shock lung" is the associated massive fall in arterial blood pressure and the prevention of the lung pathology by pretreatment with pharmacologic doses of an anti-inflammatory steroid, methylprednisolone. Evidence is also presented that suggests that the DEHP inadvertently administered to humans during transfusions is also in a solubilized state in the plasma.

In the previous progress report of October 24, 1973 the development of a detergent-solubilized dosage form of DEHP and preliminary observations of its pulmonary pathology were described. Recent progress has dealt with an in-depth study of this pathologic effect. Information in the following areas has been developed:

1. Light and electron microscopy of lung damage.
2. Pulmonary effects of DEHP solubilized in various detergents.
3. Effects on blood pressure.
4. Effects on red and white blood cells.
5. Protection against lung effects by steroids.
6. Relevance of detergent-solubilized DEHP by comparison with kinetics of disappearance of naturally migrated DEHP in patients transfused with blood products that were prepared and stored in vinyl plastic bags.

Some of this above information has already been submitted for publication in the biomedical literature. One paper has been accepted by the Journal of Toxicology and Applied Pharmacology and is currently in press. A second has been submitted to Lancet. Copies of both manuscripts are enclosed with this report.

1. Light and Electron Microscopy

The intravenous administration of 250 mg/kg of DEHP solubilized in 13.3% aqueous Tween 80 was found to be approximately an LD₅₀ dose in Wistar albino rats. Treated animals had obvious respiratory difficulty within minutes after an injection and within 5-15 minutes were markedly cyanotic. Deaths were usually seen within 90-120 minutes. Animals that survived beyond 2 hours appeared grossly to have recovered from the respiratory insult. At autopsy the lungs of animals that died appeared grossly enlarged and hemorrhagic. Lung weights were markedly elevated. Control injections of the detergent vehicle alone produced no symptoms of respiratory distress or deaths. In addition, these lungs were not different in gross appearance or weight from those of non-injected rats.

Under the light microscope (160 x) the inter-alveolar septa of lungs from DEHP-treated rats appeared greatly distended and engorged with abnormal numbers of polymorphonuclear leukocytes (PMN's). This abnormal pathology could be seen at doses of solubilized DEHP as low as 50 mg/kg. It was observable as early as 15 minutes after injection and in animals that survived the initial dose the pulmonary histology was still markedly abnormal 50 hours after the injection. The alveolitis and associated edema was quite similar in appearance to the

pathologic state in man that has been described as "shock lung". The cause of this type of lung pathology in man is not currently understood but is known to be associated with the transfusion of large numbers of blood units and with endotoxin shock.

At the electron microscopic level the picture was once again highly suggestive of "shock lung". Large numbers of PMN's were found margined on the pulmonary endothelium. Significant numbers of these PMN's were found to be undergoing degranulation and the endothelial cells were seen to be separating and allowing the leakage of fluid into the interstitium. In areas the epithelial lining cells were also found to be rupturing and allowing leakage of blood into the alveolar spaces. One additional feature not usually seen in shock lung was the deformation of the red blood cells from the normally appearing biconcave disks to a more spherical, swollen shape.

2. Pulmonary Effects of DEHP Solubilized in Other Detergents

Experiments were undertaken to determine if DEHP, solubilized in detergents other than Tween 80, also resulted in comparable lung damage or if the effect was specific to the use of Tween 80. Comparable lethality and lung pathology were seen when DEHP was solubilized in two other types of Tween detergents (Tween 20

and Tween 60) as well as in polyethylene glycol 400 (Carbowax 400) or in a polyhydroxylated vegetable oil (Emulphor 620). Thus, the toxic effect of the DEHP appears to be related to its physical state as a solubilized molecule rather than to an interaction with any specific detergent. It should also further be emphasized that the injection of any of the detergent vehicles alone resulted in no respiratory distress, lung pathology or lethality.

3. Effects on Blood Pressure

The similarity of the lung pathology to previously described "shock lung" at the light and electron microscopic levels has previously been mentioned. Another well-described characteristic of "shock lung" is a massive fall in mean arterial pressure. In order to further characterize the DEHP effect, experiments were undertaken in the rat to determine the effect of intravenous doses of solubilized DEHP on blood pressure. At doses near the lethal level (80-200 mg/kg i.v.) massive falls in mean arterial pressure were noted, but only after a time lag of 1-2 minutes; this initial period was then followed by a precipitous drop. This observation would tend to rule out a direct effect of the solubilized DEHP on vascular smooth muscle and would

suggest a time-dependent release of a potent vasoactive material from some intracellular site. When blood pressure fell below approximately 30 mm Hg, death invariably ensued. If the animal survived the dose, the blood pressure slowly returned to control levels over a 20-30 minute period.

At doses of solubilized DEHP of 40 mg/kg at which no deaths were observed, the mean fall in blood pressure was 18-27 mm Hg. This fall in blood pressure was all the more dramatic since injection of the Tween 80 vehicle alone resulted in a hypertensive response of 24-45 mm Hg. Thus, the net fall in blood pressure with DEHP was the resultant of the hypertensive effect of the vehicle overcome by the hypotensive effect of the DEHP per se. It is interesting to note that a second dose of DEHP in the same animal resulted in no significant alteration in blood pressure. Thus, although the direct hypertensive effect of the vehicle was overcome, the hypotensive effect of the DEHP was not sufficient, under these conditions, to result in a net fall in blood pressure. This observation of a decreased effect of a subsequent dose of DEHP is interpreted to indicate that the intracellular pool of vasoactive material that is presumably released by the DEHP is considerably depleted by the first dose and has not had a chance to be

regenerated over the time-course of the experiment.

4. Effect on Red and White Blood Cells

Another well-recognized aspect of "shock lung" is an increase in the number of circulating leukocytes in the blood. This leukocytosis has also been confirmed in the rats treated with solubilized DEHP. The white blood cell count was observed to increase approximately 2-3 fold within 60-90 minutes at doses of 100 mg/kg. Since the studies described above on the electron microscopic picture of the lungs of treated animals revealed a marked accumulation of leukocytes (PMN's) in the interalveolar septa, experiments are currently underway to directly measure the uptake of PMN's from the blood by the lungs. However, no definitive results are available at the time of this report.

During the experiments on white blood cell counts, an intravascular hemolysis of red blood cells (RBC's) was noted. No effect was observed with injection of the vehicle alone. Following the solubilized DEHP no hemolysis was noted for approximately 20-30 minutes; this was followed by an increasing degree of hemolysis over time. This lag period for in vivo hemolysis once again argues against a direct hemolytic effect of the solubilized DEHP and can be interpreted to represent the release or activation of intracellular hemolytic factors.

Consistent with this interpretation is the observed lack of a hemolytic effect of solubilized DEHP incubated with RBC's in vitro. In fact, under in vitro conditions the vehicle alone resulted in hemolysis which was prevented by the presence of DEHP. The in vivo hemolysis was shown to result from an increased fragility of the RBC's. Washed red cells isolated from DEHP-treated rats exhibited marked hemolysis during further in vitro incubation in saline at 37°C.

These results of increased fragility and hemolysis of RBC's is consistent with the altered morphological appearance of these cells in the electron microscope, as was described above. Although this feature has not previously been recognized as part of the "shock lung" syndrome, it may represent an additional aspect of DEHP toxicity.

5. Protection by Steroids

Since the pathologic alterations and lethality of "shock lung" are known to be prevented by pretreatment with anti-inflammatory steroids, experiments were undertaken in which one of these potent agents, methylprednisolone (solu-medrol), was tested for its ability to protect against the DEHP toxicity detailed above. Methylprednisolone, at a dose of 30 mg/kg i.v. given 15 minutes prior to the administration of the solubilized

DEHP completely prevented the gross lung pathology and reduced the acute lethality. Lung weights were not significantly different from vehicle-injected or non-injected controls. Lethality was reduced from 100% of the animals given DEHP alone (death occurring within 36.3 ± 5.9 minutes) to 33% of the pre-treated animals (with these dying at a mean time of 61.7 minutes). Thus, once again the symptoms induced by solubilized DEHP are entirely consistent with other models of "shock lung".

6. Relevance of Detergent-solubilized DEHP to that Occurring by Natural Migration from Vinyl Plastic Surfaces into Biologic Products

DEHP is a highly water-insoluble oil. This very property is what makes surprising the high levels of this chemical in blood and blood products stored in vinyl plastic bags (0.25 mg/100 ml/24 hours of storage in whole blood at 4°C to 28.0 mg/100 ml/24 hours of storage in blood platelets at 23°C). Although the appropriate physical/chemical tests have not yet been carried out on DEHP-contaminated biological solutions to determine the actual physical state of the oil, certain kinetic studies carried out in this laboratory are consistent with it existing in a solubilized state.

Previous work in rats reviewed in an earlier progress report, has shown that emulsified droplets of DEHP are cleared from the blood at a bi-exponential rate whereas solubilized DEHP is cleared at a single exponential rate which is approximately equal to the slower of the two rates seen with the emulsified dosage form. Additional experiments, also previously reviewed, have allowed the conclusion that the rapid rate seen with emulsified DEHP is due to the uptake of particulate droplets of the oil by the liver. The slow rate seen with both the emulsified and solubilized DEHP is presumably due to the disappearance of solubilized DEHP by the natural processes of metabolism and urinary excretion. In recent experiments (manuscript submitted as part of report) the rate of disappearance of DEHP has been followed in humans who had inadvertently received large amounts of this chemical during transfusion of platelets prepared and stored in vinyl plastic bags. The disappearance was found to be mono-exponential and to have a rate approximately equal to the slow rate of disappearance of the emulsified DEHP and the mono-exponential rate of solubilized DEHP in rats. Thus, the kinetic behavior of DEHP in man following natural migration from a vinyl plastic surface is entirely consistent with the phthalate existing in a solubilized state. This lends support to the over-all approach represented

in this report of being concerned with the toxicologic potential of solubilized DEHP.